

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 38/00, C07K 5/00, 7/00, 17/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/20885</b> <b>(43) International Publication Date:</b> 22 May 1998 (22.05.98)
<b>(21) International Application Number:</b> PCT/US96/18374 <b>(22) International Filing Date:</b> 13 November 1996 (13.11.96) <b>(71) Applicant (for all designated States except US):</b> UNIVERSITY OF CINCINNATI [US/US]; Mail Location 0829, G-13 Wherry Hall, Cincinnati, OH 45267-0829 (US). <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> BALASUBRAMANIAM, Ambikaipakan [US/US]; 2706 Lawyers Pointe Drive, Cincinnati, OH 45244 (US). <b>(74) Agent:</b> TSAO, Y., Rocky; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> ANALOGS OF PEPTIDE YY AND USES THEREOF  <b>(57) Abstract</b>  The invention provides analogs of PYY. The invention also provides compositions and methods useful for controlling biological activities such as cell proliferation, nutrient transport, lipolysis, and intestinal water and electrolyte secretion.		

BEST AVAILABLE COPY

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

- 1 -

ANALOGS OF PEPTIDE YY AND USES THEREOFStatement as To Federally Sponsored Research

This invention was made in part with Government  
5 funding and the Government therefore has certain rights  
in the invention.

Background of the Invention

This invention relates to peptide derivatives  
which are useful as therapeutic agents in the treatment  
10 of gastroenterological disorders.

Peptide YY (PYY) is a 36-residue peptide amide  
isolated originally from porcine intestine, and localized  
in the endocrine cells of the gastrointestinal tract and  
pancreas (Tatemoto et al. *Proc. Natl. Acad. Sci.* 79:2514,  
15 1982). Peptide YY has N-terminal and C-terminal tyrosine  
amides; accordingly, these two tyrosines give PYY its  
name (Y represents the amino acid tyrosine in the peptide  
nomenclature). In addition PYY shares a number of  
central and peripheral regulatory roles with its  
20 homologous peptide neuropeptide Y (NPY), which was  
originally isolated from porcine brain (Tatemoto, *Proc.*  
*Natl. Acad. Sci.* 79:5485, 1982). In contrast with the  
cellular location of PYY, NPY is present in submucous and  
myenteric neurons which innervate the mucosal and smooth  
25 muscle layers, respectively (Ekblad et al. *Neuroscience*  
20:169, 1987). Both PYY and NPY are believed to inhibit  
gut motility and blood flow (Laburthe, *Trends Endocrinol.*  
*Metab.* 1:168, 1990), and they are also thought to  
attenuate basal (Cox et al. *Br. J. Pharmacol.* 101:247,  
30 1990; Cox et al. *J. Physiol.* 398:65, 1988; Cox et al.  
*Peptides* 12:323, 1991; Friel et al. *Br. J. Pharmacol.*  
88:425, 1986) and secretagogue-induced intestinal  
secretion in rats (Lundberg et al. *Proc. Natl. Acad. Sci*  
*USA* 79:4471, 1982; Playford et al. *Lancet* 335:1555, 1990)  
35 and humans (Playford et al. *supra*), as well as stimulate

- 2 -

net absorption (MacFadyen et al. *Neuropeptides* 7:219, 1986). Furthermore, plasma PYY levels have been reported to be elevated in several diseases that cause diarrhea (Adrian et al. *Gastroenterology* 89:1070, 1985). Taken  
5 together, these observations suggest that PYY and NPY are released into the circulation after a meal (Adrian et al. *Gastroenterology* 89:1070, 1985; Balasubramaniam et al. *Neuropeptides* 14:209, 1989), and thus may play a physiological role in regulating intestinal secretion and  
10 absorption, serving as natural inhibitors of diarrhea.

A high affinity PYY receptor system which exhibits a slightly higher affinity for PYY than NPY has been characterized in rat intestinal epithelia (Laburthe et al. *Endocrinology* 118:1910, 1986; Laburthe, *Trends*  
15 *Endocrinol. Metab. supra*) and shown to be negatively coupled to adenylate cyclase (Servin et al. *Endocrinology* 124:692, 1989). Consistently, PYY exhibited greater antiseecretory potency than NPY in voltage clamped preparations of rat small intestine (Cox et al. *J.*  
20 *Physiol. supra*), while C-terminal fragments of NPY were found to be less effective in their antiseecretory potency than PYY (Cox et al. *Br. J. Pharmacol. supra*). Structure-activity studies using several partial sequences have led to the  
25 identification of PYY(22-36) as the active site for interacting with intestinal PYY receptors (Balasubramaniam et al. *Pept. Res.* 1:32, 1988).

In addition, PYY has been implicated in a number of physiological activities including nutrient uptake  
30 (see, e.g., Bilcheik et al. *Digestive Disease Week* 506:623, 1993), cell proliferation (see, e.g., Laburthe, *Trends Endocrinol. Metab.* 1:168, 1990; Voisin et al. *J. Biol. Chem.*, 1993), lipolysis (see, e.g., Valet et al., *J. Clin. Invest.* 85:291, 1990), and vasoconstriction

- 3 -

(see, e.g., Lundberg et al., *Proc. Natl. Acad. Sci., USA* 79: 4471, 1982).

The amino acid sequences of porcine and human PYY are as follows:

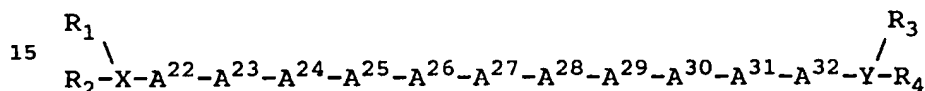
5 porcine PYY YPAKPEAPGEDASPEELSRYYASLRHYLNLVTRQRY (SEQ. ID. NO. 1)

human PYY YPIKPEAPGEDASPEELNRYASLRHYLNLVTRQRY (SEQ. ID. NO. 2)

The amino acid sequence for dog PYY and rat is the same  
10 as porcine PYY.

### Summary of the Invention

In one aspect, the present invention features novel analogs of peptide YY of the formula:



(Formula I)

wherein

20 X is a chain of 0-5 amino acids, inclusive, the N-terminal one of which is bonded to R<sub>1</sub> and R<sub>2</sub>;

Y is a chain of 0-4 amino acids, inclusive, the C-terminal one of which is bonded to R<sub>3</sub> and R<sub>4</sub>;

25 R<sub>1</sub> is H, C<sub>1</sub>-C<sub>12</sub> alkyl (e.g., methyl), C<sub>6</sub>-C<sub>18</sub> aryl (e.g., phenyl, naphthaleneacetyl), C<sub>1</sub>-C<sub>12</sub> acyl (e.g., formyl, acetyl, and myristoyl), C<sub>7</sub>-C<sub>18</sub> aralkyl (e.g., benzyl), or C<sub>7</sub>-C<sub>18</sub> alkaryl (e.g., p-methylphenyl);

30 R<sub>2</sub> is H, C<sub>1</sub>-C<sub>12</sub> alkyl (e.g., methyl), C<sub>6</sub>-C<sub>18</sub> aryl (e.g., phenyl, naphthaleneacetyl), C<sub>1</sub>-C<sub>12</sub> acyl (e.g., formyl, acetyl, and myristoyl), C<sub>7</sub>-C<sub>18</sub> aralkyl (e.g., benzyl), or C<sub>7</sub>-C<sub>18</sub> alkaryl (e.g., p-methylphenyl);

A<sup>22</sup> is an aromatic amino acid, Ala, Aib, Anb, N-Me-Ala, or is deleted;

- 4 -

- Me- A<sup>23</sup> is Ser, Thr, Ala, Aib, N-Me-Ser, N-Me-Thr, N  
Ala, or is deleted;
- Anb, A<sup>24</sup> is Leu, Ile, Val, Trp, Gly, Nle, Nva, Aib,  
N-Me-Leu, or is deleted;
- 5 ε- A<sup>25</sup> is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-  
NH-R (where R is H, a branched or straight  
chain C<sub>1</sub>-C<sub>10</sub> alkyl group, or an aryl group),  
Orn, or is deleted;
- 10 A<sup>26</sup> is Ala, His, Thr, 3-Me-His, 1-Me-His, β-  
pyroazolylalanine, N-Me-His, Arg, Lys, homo-  
Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is  
H, a branched or straight chain C<sub>1</sub>-C<sub>10</sub> alkyl  
group, or an aryl group), Orn, or is deleted;
- 15 A<sup>27</sup> is an aromatic amino acid other than Tyr;  
A<sup>28</sup> is Leu, Ile, Val, Trp, Nle, Nva, Aib, Anb, or  
N-Me-Leu;
- A<sup>29</sup> is Asn, Ala, Gln, Gly, Trp, or N-Me-Asn;
- A<sup>30</sup> is Leu, Ile, Val, Trp, Nle, Nva, Aib, Anb, or  
N-Me-Leu;
- 20 A<sup>31</sup> is Val, Leu, Ile, Trp, Nle, Nva, Aib, Anb, or  
N-Me-Val;
- A<sup>32</sup> is Thr, Ser, N-Me-Ser, N-Me-Thr, or D-Trp;
- R<sub>3</sub> is H, C<sub>1</sub>-C<sub>12</sub> alkyl (e.g., methyl), C<sub>6</sub>-C<sub>18</sub> aryl  
(e.g., phenyl, naphthaleneacetyl), C<sub>1</sub>-C<sub>12</sub>  
25 acyl (e.g., formyl, acetyl, and myristoyl),  
C<sub>7</sub>-C<sub>18</sub> aralkyl (e.g., benzyl), or C<sub>7</sub>-C<sub>18</sub>  
alkaryl (e.g., p-methylphenyl); and
- R<sub>4</sub> is H, C<sub>1</sub>-C<sub>12</sub> alkyl (e.g., methyl), C<sub>6</sub>-C<sub>18</sub> aryl  
(e.g., phenyl, naphthaleneacetyl), C<sub>1</sub>-C<sub>12</sub>  
30 acyl (e.g., formyl, acetyl, and myristoyl),  
C<sub>7</sub>-C<sub>18</sub> aralkyl (e.g., benzyl), or C<sub>7</sub>-C<sub>18</sub>  
alkaryl (e.g., p-methylphenyl), or a  
pharmaceutically acceptable salt thereof.

- 5 -

In preferred embodiments, A<sup>27</sup> is Phe, Nal, Bip, Pcp, Tic, Trp, Bth, Thi, or Dip.

In preferred embodiments X is A<sup>17</sup>-A<sup>18</sup>-A<sup>19</sup>-A<sup>20</sup>-A<sup>21</sup> wherein

5 A<sup>17</sup> is Cys, Leu, Ile, Val, Nle, Nva, Aib, Anb, or N-Me-Leu;

A<sup>18</sup> is Cys, Ser, Thr, N-Me-Ser, or N-Me-Thr;

ε- A<sup>19</sup> is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-  
10 NH-R (where R is H, a branched or straight chain C<sub>1</sub>-C<sub>10</sub> alkyl group, or C<sub>6</sub>-C<sub>18</sub> aryl group), Cys, or Orn;

A<sup>20</sup> is an aromatic amino acid, or Cys; and

15 A<sup>21</sup> is an aromatic amino acid, Cys, or a pharmaceutically acceptable salt thereof. In yet other preferred embodiments, Y is A<sup>33</sup>-A<sup>34</sup>-A<sup>35</sup>-A<sup>36</sup> wherein

ε- A<sup>33</sup> is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-  
20 NH-R (where R is H, a branched or straight chain C<sub>1</sub>-C<sub>10</sub> alkyl group, or an aryl group), Cys, or Orn;

A<sup>34</sup> is Cys, Gln, Asn, Ala, Gly, N-Me-Gln, Aib, or Anb;

ε- A<sup>35</sup> is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-  
25 NH-R (where R is H, a branched or straight chain C<sub>1</sub>-C<sub>10</sub> alkyl group, or an aryl group), Cys, or Orn; and

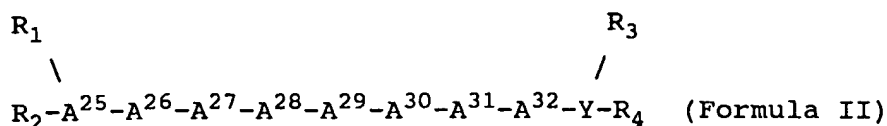
A<sup>36</sup> is an aromatic amino acid, Cys or a pharmaceutically acceptable salt thereof.

Preferably, the compound has the formula: N-α-Ac-  
30 Ala-Ser-Leu-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH<sub>2</sub> (SEQ. ID. NO. 3), H-Ala-Ser-Leu-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH<sub>2</sub> (SEQ. ID. NO. 4), N-α-Ac-Ala-Ser-Leu-Arg-His-Trp-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH<sub>2</sub> (SEQ. ID. NO. 5), N-α-Ac-Ala-Ser-Leu-Arg-His-  
35 Thi-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH<sub>2</sub> (SEQ. ID. NO.

- 6 -

6), N- $\alpha$ -Ac-Tyr-Ser-Leu-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH<sub>2</sub> (SEQ. ID. NO. 7) or a pharmaceutically acceptable salt thereof.

In another aspect the invention features novel  
5 analogs of peptide YV of the formula:



wherein

- 10 the N-terminal amino acid is bonded to R<sub>1</sub> and R<sub>2</sub>;  
Y is a chain of 0-4 amino acids, inclusive the C-terminal one of which is bonded to R<sub>3</sub> and R<sub>4</sub>;  
R<sub>1</sub> is H, C<sub>1</sub>-C<sub>12</sub> alkyl (e.g., methyl), C<sub>6</sub>-C<sub>18</sub> aryl (e.g., phenyl, naphthaleneacetyl), C<sub>1</sub>-C<sub>12</sub> acyl (e.g., formyl, acetyl, and myristoyl), C<sub>7</sub>-C<sub>18</sub> aralkyl (e.g., benzyl), or C<sub>7</sub>-C<sub>18</sub> alkaryl (e.g., *p*-methylphenyl);  
15 R<sub>2</sub> is H, C<sub>1</sub>-C<sub>12</sub> alkyl (e.g., methyl), C<sub>6</sub>-C<sub>18</sub> aryl (e.g., phenyl, naphthaleneacetyl), C<sub>1</sub>-C<sub>12</sub> acyl (e.g., formyl, acetyl, and myristoyl), C<sub>7</sub>-C<sub>18</sub> aralkyl (e.g., benzyl), or C<sub>7</sub>-C<sub>18</sub> alkaryl (e.g., *p*-methylphenyl);  
20 A<sup>25</sup> is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys- $\epsilon$ -NH-R (where R is H, a branched or straight chain C<sub>1</sub>-C<sub>10</sub> alkyl group, or an aryl group), Orn, or is deleted;  
25 A<sup>26</sup> is Ala, His, Thr, 3-Me-His, 1-Me-His,  $\beta$ -pyroglutamylalanine, N-Me-His, Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys- $\epsilon$ -NH-R (where R is H, a branched or straight chain C<sub>1</sub>-C<sub>10</sub> alkyl group, or an aryl group), Orn, or is deleted;  
30 A<sup>27</sup> is an aromatic amino acid;  
A<sup>28</sup> is Leu, Ile, Val, Trp, Nle, Nva, Aib, Anb, or N-Me-Leu;



- 7 -

A<sup>29</sup> is Asn, Ala, Gln, Gly, Trp, or N-Me-Asn;

A<sup>30</sup> is Leu, Ile, Val, Trp, Nle, Nva, Aib, Anb, or N-Me-Leu;

A<sup>31</sup> is Val, Ile, Trp, Nva, Aib, Anb, or N-Me-Val;

5 A<sup>32</sup> is Thr, Ser, N-Me-Ser, N-Me-Thr, or D-Trp;

R<sub>3</sub> is H, C<sub>1</sub>-C<sub>12</sub> alkyl (e.g., methyl), C<sub>6</sub>-C<sub>18</sub> aryl (e.g., phenyl, naphthaleneacetyl), C<sub>1</sub>-C<sub>12</sub> acyl (e.g., formyl, acetyl, and myristoyl), C<sub>7</sub>-C<sub>18</sub> aralkyl (e.g., benzyl), or C<sub>7</sub>-C<sub>18</sub> alkaryl (e.g., p-methylphenyl); and

10

R<sub>4</sub> is H, C<sub>1</sub>-C<sub>12</sub> alkyl (e.g., methyl), C<sub>6</sub>-C<sub>18</sub> aryl (e.g., phenyl, naphthaleneacetyl), C<sub>1</sub>-C<sub>12</sub> acyl (e.g., formyl, acetyl, and myristoyl), C<sub>7</sub>-C<sub>18</sub> aralkyl (e.g., benzyl), or C<sub>7</sub>-C<sub>18</sub> alkaryl (e.g., p-methylphenyl), or a pharmaceutically acceptable salt thereof.

15

In preferred embodiments A<sup>27</sup> is Phe, Nal, Bip, Pcp, Tic, Trp, Bth, Thi, or Dip.

In preferred embodiments Y is A<sup>33</sup>-A<sup>34</sup>-A<sup>35</sup>-A<sup>36</sup>

20 wherein

A<sup>33</sup> is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-  
 ε- NH-R (where R is H, a branched or straight  
 chain C<sub>1</sub>-C<sub>10</sub> alkyl group, or C<sub>6</sub>-C<sub>18</sub> aryl  
 group), Cys, or Orn;

25 A<sup>34</sup> is Gln, Asn, Ala, Gly, N-Me-Gln, Aib, Cys, or Anb;

A<sup>35</sup> is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-  
 ε- NH-R (where R is H, a branched or straight  
 chain C<sub>1</sub>-C<sub>10</sub> alkyl group, or C<sub>6</sub>-C<sub>18</sub> aryl  
 group), Cys, or Orn; and

30

A<sup>36</sup> is an aromatic amino acid, Cys, or a pharmaceutically acceptable salt thereof.

Preferably, the compound has the formula N-α-Ac-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH<sub>2</sub> (SEQ.

35 ID. NO. 8).

- 8 -

In another aspect, the invention features novel dimeric analogs of peptide YY. The dimer may be formed by either including two peptides of Formula I, two peptides of Formula II, or one peptide of Formula I and  
5 one peptide of Formula II. In one embodiment, the dimer is formed by utilizing a dicarboxylic acid linker capable of binding to a free amine, either primary or secondary, located within each peptide. See, e.g., R. Vavrek and J. Stewart, Peptides: Structure and Function 381-384 (Pierce  
10 Chemical Co. 1983). Examples of suitable dicarboxylic acid linkers are succinic acid, glutamic acid, and phthalic acid. In other embodiments, the dimer is formed by utilizing an amino acid linker capable of binding to a free amine group of one peptide and a free carboxyl group  
15 of the other peptide. Preferably, the amino acid linker is a non  $\alpha$ -amino acid. Examples of suitable amino acid linkers are amino-caproic acid and amino-valeric acid. In yet another embodiment, the dimer is formed by a disulfide bridge between cysteines located within each  
20 peptide. See, e.g., M. Berngtowicz and G. Piatsueda, Peptides: Structure and Function 233-244 (Pierce Chemical Co. 1985); F. Albericio, et al., Peptides 1990. 535 (ESCOM 1991).

In yet another aspect, the invention features  
25 analogs of Formula I or Formula II having at least one pseudopeptide bond between amino acid residues. By "pseudopeptide bond" is meant that the carbon atom participating in the bond between two residues is reduced from a carbonyl carbon to a methylene carbon, i.e.,  $\text{CH}_2$ -  
30  $\text{NH}$ ; or less preferably that of  $\text{CO-NH}$  is replaced with any of  $\text{CH}_2\text{-S}$ ,  $\text{CH}_2\text{-CH}_2$ ,  $\text{CH}_2\text{-O}$ , or  $\text{CH}_2\text{-CO}$ . A pseudopeptide bond is symbolized herein by " $\Psi$ ". Preferably, the pseudopeptide bonds are located between one or more amino acid residues, e.g.,  $\text{A}^{28}\Psi\text{A}^{29}$ ,  $\text{A}^{29}\Psi\text{A}^{30}$ ,  $\text{A}^{30}\Psi\text{A}^{31}$ ,  
35  $\text{A}^{31}\Psi\text{A}^{32}$ ,  $\text{A}^{32}\Psi\text{A}^{33}$ ,  $\text{A}^{33}\Psi\text{A}^{34}$ ,  $\text{A}^{34}\Psi\text{A}^{35}$ , or  $\text{A}^{35}\Psi\text{A}^{36}$ . In addition,

- 9 -

such pseudopeptide bond analogs can be used to form dimeric analogs as is described above. A detailed discussion of the chemistry of pseudopeptide bonds is given in Coy et al. (1988) *Tetrahedron* 44:835-841.

5 In yet another aspect, the invention features radiolabeled analogs of Formula I and Formula II. Preferably, the analogs have a tyrosine iodinated on the phenyl ring at carbon position 3 or 5. The radioactive iodine is preferably  $I^{125}$  or  $I^{123}$ . An example of the  
10 chemistry associated with iodinated tyrosine residues within peptides, see European Patent Application 0389180, herein incorporated by reference. Accordingly, radiolabeled PYY analogs can be used for imaging PYY  
15 receptors, e.g., for imaging cells containing PYY receptors.

The symbol X, Y, Z,  $A^{22}$ ,  $A^{23}$ ,  $A^{24}$ , and the like; and Ser, Leu or the like, as found in a peptide sequence herein stands for an amino acid residue, i.e.,  $=N-CH(R)-CO-$  when it is at the N-terminus, or  $-NH-CH(R)-CO-N=$  when  
20 it is at C-terminus, or  $-NH-CH(R)-CO-$  when it is not at the N- or C-terminus, where R denotes the side chain (or identifying group) of an amino acid or its residue. For example, R is  $-CH_2COOH$  for Asp, R is  $-H$  for Gly, R is  $-CH_2OH$  for Ser, R is  $-CH_3$  for Ala and R is  $-CH_2CH_2CH_2CH_2NH_2$   
25 for Arg. Also, when the amino acid residue is optically active, it is the L-form configuration that is intended unless the D-form is expressly designated.

As set forth above and for convenience in describing this invention, the conventional and  
30 nonconventional abbreviations for the various amino acids are used. They are familiar to those skilled in the art; but for clarity are listed below. All peptide sequences mentioned herein are written according to the usual convention whereby the N-terminal amino acid is on the  
35 left and the C-terminal amino acid is on the right. A

- 10 -

short line between two amino acid residues indicates a peptide bond.

	Asp	=	D	=	Aspartic Acid
	Ala	=	A	=	Alanine
5	Arg	=	R	=	Arginine
	Asn	=	N	=	Asparagine
	Cys	=	C	=	Cysteine
	Gly	=	G	=	Glycine
	Glu	=	E	=	Glutamic Acid
10	Gln	=	Q	=	Glutamine
	His	=	H	=	Histidine
	Ile	=	I	=	Isoleucine
	Leu	=	L	=	Leucine
	Lys	=	K	=	Lysine
15	Met	=	M	=	Methionine
	Phe	=	F	=	Phenylalanine
	Pro	=	P	=	Proline
	Ser	=	S	=	Serine
	Thr	=	T	=	Threonine
20	Trp	=	W	=	Tryptophan
	Tyr	=	Y	=	Tyrosine
	Val	=	V	=	Valine
	Orn	=		=	Ornithine
	Nal	=		=	2-napthylalanine
25	Nva	=		=	Norvaline
	Nle	=		=	Norleucine
	Thi	=		=	2-thienylalanine
	Pcp	=		=	4-chlorophenylalanine
	Bth	=		=	3-benzothienylalanine
30	Bip	=		=	4,4'-biphenylalanine
	Tic	=		=	tetrahydroisoquinoline-3-carboxylic acid
	Aib	=		=	aminoisobutyric acid
	Anb	=		=	$\alpha$ -aminonormalbutyric acid

- 11 -

Dip = 2,2-diphenylalanine

Thz = 4-Thiazolylalanine

The compounds of the present invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, trifluoroacetic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids, such as hydrohalic acids, e.g., hydrochloric acid, sulfuric acid, or phosphoric acid and the like.

In another aspect, the invention features one of the above compounds and a pharmaceutically acceptable carrier substance in a therapeutic composition capable of decreasing excess intestinal water and electrolyte secretion.

In preferred embodiments, the composition is in the form of a liquid, pill, tablet, or capsule for oral administration; a liquid capable of being administered nasally as drops or spray or a liquid for intravenous, subcutaneous, parenteral, intraperitoneal or rectal administration. The therapeutic composition can also be in the form of an oil emulsion or dispersion in conjunction with a lipophilic salt such as pamoic acid, or in the form of a biodegradable sustained-release composition for subcutaneous or intramuscular administration. For maximum efficacy, zero-order release is desired.

In another aspect the invention features, a method for decreasing excess intestinal water and electrolyte secretion in a mammal, the method comprising administering to the mammal, e.g., a human, a

- 12 -

therapeutically effective amount of the above mentioned compounds.

In addition, the invention features a method of regulating cell proliferation in a mammal, the method comprising administering to the mammal a therapeutically effective amount of the composition of the above mentioned compounds. Preferably, the method regulates the proliferation of an intestinal cell.

The invention also features methods for increasing nutrient transport, regulating lipolysis, and regulating blood flow in a mammal, the methods comprising administering to the mammal a therapeutically effective amount of the above mentioned compositions.

The compounds of the invention exhibit a broad range of biological activities related to their antisecretory and antimotility properties. The compounds are believed to suppress gastrointestinal secretions by direct interaction with epithelial cells or, perhaps, by inhibiting secretion of hormones or neurotransmitters which stimulate intestinal secretion. The compounds of the invention may also control intestinal blood flow which in turn may modulate intestinal hydrostatic pressure in favor of net water absorption.

The compounds of the invention are especially useful in the treatment of any number of gastrointestinal disorders (see e.g., *Harrison's Principles of Internal Medicine*, McGraw-Hill Inc., New York, 12th Ed.) that are associated with excess intestinal electrolyte and water secretion as well as decreased absorption, e.g., infectious (e.g., viral or bacterial) diarrhea, inflammatory diarrhea, short bowel syndrome, or the diarrhea which typically occurs following surgical procedures, e.g., ileostomy. Examples of infectious diarrhea include, without limitation, acute viral diarrhea, acute bacterial diarrhea (e.g., salmonella,

- 13 -

campylobacter, and clostridium or due to protozoal infections), or traveller's diarrhea (e.g., Norwalk virus or rotavirus). Examples of inflammatory diarrhea include, without limitation, malabsorption syndrome, 5 tropical spue, chronic pancreatitis, Crohn's disease, diarrhea, and irritable bowel syndrome. It has also been discovered that the peptides of the invention can be used to treat an emergency or life-threatening situation involving a gastrointestinal disorder, e.g., after 10 surgery or due to cholera. Furthermore, the compounds of the invention can be used to treat patients suffering from Acquired Immune Deficiency Syndrome (AIDS), especially during cachexia.

The compounds of the invention are also useful for 15 inhibiting small intestinal fluid and electrolyte secretion, augmenting nutrient transport -- as well as increasing cell proliferation -- in the gastrointestinal tract, regulating lipolysis in, e.g, adipose tissue, and regulating blood flow in a mammal.

20 The compounds of the invention are advantageous because they are truncated versions of the natural PYY peptide; thus, the shorter peptide not only facilitates easier synthesis and purification of the compounds, but also improves and reduces manufacturing procedures and 25 expenses. Moreover, a shorter PYY compound is advantageous because such peptides will interact solely with PYY receptors and not with homologous receptors such as NPY Y1 and Y3; thus, minimizing unwanted agonist or antagonist side reactions.

30 Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

- 14 -

Detailed Description

The drawings will first be described.

Drawings

FIG. 1 shows a semipreparative reversed phase  
5 chromatogram of N- $\alpha$ -Ac-[Phe<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 3)  
( $\approx$ 25mg) obtained by HF cleavage. Conditions: Vydac C18  
semipreparative column (250 X 10mm, 300 Å pore size, 10  
micron particle size); flow rate 4.7 ml/min; fractions 1,  
2, 3, and 4 were collected and analyzed by analytical  
10 chromatography. The homogeneous fractions (1-3) were  
combined and dried in a speed vac.

FIG. 2 shows a graph of the inhibition of <sup>125</sup>I-  
PYY binding to rat jejunal membranes by increasing  
concentrations of PYY (SEQ. ID. NO. 1), PYY(22-36) (SEQ.  
15 ID. NO. 10), [Im-DNP-His<sup>26</sup>]PYY (SEQ. ID. NO. 9),  
[Ala<sup>32</sup>]PYY(22-36) (SEQ. ID. NO. 11), [Ala<sup>23,32</sup>]PYY(22-36)  
(SEQ. ID. NO. 12), [Glu<sup>28</sup>]PYY(22-36) (SEQ. ID. NO. 13), N-  
 $\alpha$ -Ac-PYY(22-36) (SEQ. ID. NO. 14), N- $\alpha$ -Ac-[p.Cl-  
Phe<sup>28</sup>]PYY(22-36) (SEQ. ID. NO. 15), N- $\alpha$ -Ac-[Glu<sup>26</sup>]PYY(22-  
20 36) (SEQ. ID. NO. 16), N- $\alpha$ -Ac-[Phe<sup>27</sup>]PYY(22-36) (SEQ. ID.  
NO. 3), N- $\alpha$ -Ac-[N-Me-Tyr<sup>26</sup>]PYY(22-36) (SEQ. ID. NO. 17), N-  
 $\alpha$ -Myristoyl-PYY(22-36) (SEQ. ID. NO. 18), N- $\alpha$ -  
Naphthaleneacetyl-PYY(22-36) (SEQ. ID. NO. 19), and PYY  
(22-26) (SEQ. ID. NO. 10).

25 FIGS. 3A-B show the antisecretory effects of PYY  
(SEQ. ID. NO. 1), PYY(22-36) (SEQ. ID. NO. 10) and analogs  
up one baseline short circuit current (SCC) in voltage  
clamped preparation of rat jejunum. Values of changes in  
SCC are quoted of  $\mu$ A/0.6cm<sup>2</sup>, mean  $\pm$ SEM from between 3 and  
30 7 different jejunal preparations. Peptides shown in A  
and B are denoted by the same symbol as in FIG. 2.

FIG. 4 shows a graph of the inhibition of <sup>125</sup>I-PYY  
binding to rat jejunal membranes by increasing  
concentrations of PYY, N- $\alpha$ -Ac-PYY(22-36) (SEQ. ID. NO.  
35 14), N- $\alpha$ -Ac-[Tic<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 25), N- $\alpha$ -Ac-



- 15 -

[Bip<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 22), N- $\alpha$ -Ac-[Nal<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 23), N- $\alpha$ -Ac-[Bth<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 21), N- $\alpha$ -Ac-[Phe<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 3), N- $\alpha$ -Ac-[Phe<sup>27</sup>]PYY(25-36) (SEQ. ID. NO. 26), N- $\alpha$ -Ac-[Trp<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 5), and N- $\alpha$ -Ac-[Thi<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 6).

There now follows a description of the synthesis, analysis for biological efficacy and use of the preferred embodiments of the invention. In order to determine the structural requirements necessary to elicit antisecretory effects, several analogs of the PYY active site, PYY(22-36), were synthesized and their binding and antisecretory potencies in rat jejunum were compared.

We now describe the structure, synthesis, and use of preferred embodiments of the invention.

#### STRUCTURE

The peptides of the invention have the general formula recited in the Summary of the Invention above. They all have an aromatic amino acid group at position 27 which is important for both antisecretory activity and utility as antidiarrheal compounds.

#### SYNTHESIS

The peptides of the present invention may be synthesized by any techniques that are known to those skilled in the peptide art. An excellent summary of the many techniques so available may be found in *Solid Phase Peptide Synthesis* 2nd ed. (Stewart, J.M. and Young, J. D. Pierce Chemical Company, Rockford, IL, 1984).

The peptides listed in Table 1 and Table 2 were synthesized as follows. Peptide synthesis was performed on an Applied Biosystems Model 430A synthesizer. Amino acid and sequence analyses were carried out using Waters Pico-Tag and Applied Biosystems Model 470A instruments,

- 16 -

respectively. Peptides were purified using a Waters Model 600 solvent delivery system equipped with a Model 481 Spectrophotometer and U6K injector according to standard protocols. Peptide masses were determined at the University of Michigan, Protein Chemistry Facility, Ann Arbor, Michigan according to standard methods. All Boc-L-amino acid derivatives, solvents, chemicals and the resins were obtained commercially and used without further purification.

Paramethylbenzhydroxylamine (MBHA) resin (0.45 mmol,  $-\text{NH}_2$ ) was placed in the reaction vessel of the peptide synthesizer and the protected amino acid derivatives were sequentially coupled using the program provided by the manufacturers modified to incorporate a double coupling procedure (see, e.g., Balasubramaniam et al., *Peptide Research* 1: 32, 1988). All amino acids were coupled using 2.2 equivalents of preformed symmetrical anhydrides. Arg, Gln and Asn, however, were coupled as preformed 1-hydroxybenzotriazole (HOBt) esters to avoid side reactions. At the end of the synthesis, the N- $\alpha$ -Boc group was removed and in some instances the free  $\alpha$ - $\text{NH}_2$  was acetylated by reaction with acetic anhydride (2 equivalents) and diisopropyl ethylamine until a negative ninhydrin test was obtained (Anal. Biochem. 34:595, 1970). The peptide resin (~1.0 g) was then treated with HF (10 ml) containing p-cresol (~0.8 g) for 1 h at -2 to -4 °C. The HF was evacuated and the residue was transferred to a fritted filter funnel with diethyl ether, washed repeatedly with diethyl ether, extracted with acetic acid (2 X 15 ml) and lyophilized. The crude peptides thus obtained were purified by semipreparative RP-HPLC as shown in Fig. 1.

Examples of the synthesized analogs are:

35 [im-DNP-His<sup>26</sup>]PYY YPAKPEAPGEDASPEELSRYYASLR [im-DNP-His<sup>26</sup>]YLNLVTRQRY-NH<sub>2</sub> (SEQ. ID No. 9)  
PYY(22-36)

- 17 -

- A S L R H Y L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 10)
- [Ala<sup>32</sup>]PYY  
A S L R H Y L N L V [Ala] R Q R Y-NH<sub>2</sub> (SEQ. ID No. 11)
- [Ala<sup>23,32</sup>]PYY  
5 A [Ala] L R H Y L N L V [Ala] R Q R Y-NH<sub>2</sub> (SEQ. ID No. 12)
- [Glu<sup>28</sup>]PYY(22-36)  
A S L R H Y [Glu] N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 13)
- N-α-Ac-PYY(22-36)  
N-α-Ac-A S L R H Y L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 14)
- 10 N-α-Ac[p.Cl.Phe<sup>26</sup>]PYY  
N-α-Ac-A S L R [p.Cl.Phe<sup>26</sup>] Y L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 15)
- N-α-Ac[Glu<sup>28</sup>]PYY  
N-α-Ac-A S L R H Y [Glu] N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 16)
- N-α-Ac[Phe<sup>27</sup>]PYY  
15 N-α-Ac-A S L R H [Phe] E N L V T R Q R [N-Me-Tyr]-NH<sub>2</sub> (SEQ. ID No. 3)
- N-α-Ac[N-Me-Tyr<sup>36</sup>]PYY  
N-α-Ac-A S L R H Y E N L V T R Q R [N-Me-Tyr]-NH<sub>2</sub> (SEQ. ID No. 17)
- N-α-myristoyl-PYY(22-36)  
N-α-myristoyl-A S L R H Y L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 18)
- 20 N-α-naphthaleneacetyl-PYY(22-36)  
N-α-naphthaleneacetyl -A S L R H Y L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 19)
- N-α-Ac[Phe<sup>27</sup>]PYY  
N-α-Ac-A S L R H [Phe] E N L V T R Q R [N-Me-Tyr]-NH<sub>2</sub> (SEQ. ID No. 3)
- N-α-Ac-PYY(22-36)  
25 N-α-Ac-A S L R H Y L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 20)
- N-α-Ac-[Bth<sup>27</sup>]PYY(22-36)  
N-α-Ac-A S L R H [Bth] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 21)
- N-α-Ac-[Bip<sup>27</sup>]PYY(22-36)  
N-α-Ac-A S L R H [Bip] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 22)
- 30 N-α-Ac-[Nal<sup>27</sup>]PYY(22-36)  
N-α-Ac-A S L R H [Nal] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 23)
- N-α-Ac-[Trp<sup>27</sup>]PYY(22-36)  
N-α-Ac-A S L R H [Trp] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 5)
- N-α-Ac-[Thi<sup>27</sup>]PYY(22-36)  
35 N-α-Ac-A S L R H [Thi] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 6)
- N-α-Ac-[Tic<sup>27</sup>]PYY(22-36)  
N-α-Ac-A S L R H [Tic] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 25)
- N-α-Ac-[Phe<sup>27</sup>]PYY(25-36)  
N-α-Ac-H [Phe] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 26)
- 40 N-α-Ac-[Phe<sup>27</sup>,Thi<sup>36</sup>]PYY(22-36)  
N-α-Ac-A S L R H [Phe] L N L V T R Q R [Thi]-NH<sub>2</sub> (SEQ. ID No. 27)
- N-α-Ac-[Thz<sup>26</sup>,Phe<sup>27</sup>]PYY(22-36)  
N-α-Ac-A S L R [Thz] [Phe] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 28)
- N-α-Ac-[Pcp<sup>27</sup>]PYY(22-36)  
45 N-α-Ac-A S L R H [Pcp] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 29)
- N-α-Ac-[Phe<sup>22,27</sup>]PYY(22-36)  
N-α-Ac-[Phe] S L R H [Phe] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 30)

- 18 -

- N- $\alpha$ -Ac-[Tyr<sup>22</sup>,Phe<sup>27</sup>]PYY(22-36)  
N- $\alpha$ -Ac-[Tyr] S L R H [Phe] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 7)
- N- $\alpha$ -Ac-[Trp<sup>28</sup>]PYY(22-36)  
N- $\alpha$ -Ac- A S L R H Y [Trp] N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 31)
- 5 N- $\alpha$ -Ac-[Trp<sup>30</sup>]PYY(22-36)  
N- $\alpha$ -Ac- A S L R H Y L N [Trp] V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 32)
- N- $\alpha$ -Ac-[Ala<sup>26</sup>,Phe<sup>27</sup>]PYY(22-36)  
N- $\alpha$ -Ac- A S L R [Ala] [Phe] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 33)
- 10 N- $\alpha$ -Ac-[Bth<sup>27</sup>]PYY(22-36)  
N- $\alpha$ -Ac- A S L R H [Bth] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 34)
- N- $\alpha$ -Ac-[Phe<sup>27</sup>]PYY(22-36)  
N- $\alpha$ -Ac- A S L R H [Phe] L N L V T R Q R Y-NH<sub>2</sub> (SEQ. ID No. 35)
- N- $\alpha$ -Ac-[Phe<sup>27,36</sup>]PYY(22-36)  
N- $\alpha$ -Ac- A S L R H [Phe] L N L V T R Q R [Phe]-NH<sub>2</sub> (SEQ. ID No. 36)
- 15 N- $\alpha$ -Ac-[Phe<sup>27</sup>, D-Trp<sup>32</sup>]PYY(22-36)  
N- $\alpha$ -Ac- A S L R H [Phe] L N L V [D-Trp] R Q R Y-NH<sub>2</sub> (SEQ. ID No. 37)

## ANALYSIS

### Binding Studies

Preparation of <sup>125</sup>I-PYY labeled only at Tyr<sup>36</sup> and  
 20 rat jejunal epithelial plasma membranes were performed  
 according to standard methods (see, e.g., Laburthe et al.  
*Endocrinology, supra*; Servin et al. *supra*; Voisin et al.  
*Ann. N. Y. Acad. Sci.* 611:343, 1990). Binding  
 experiments were conducted in a total volume of 0.25 ml  
 25 60 mM HEPES buffer, pH 7, containing 2% BSA, 0.1%  
 bacitracin, 5 mM MgCl<sub>2</sub> and 0.05 nM <sup>125</sup>I-PYY with or  
 without competing peptides. Bound and free peptides were  
 separated by centrifugation at 20,000 X g for 10 min.  
 Non-specific <sup>125</sup>I-PYY binding was determined in the  
 30 presence of 1  $\mu$ M unlabeled PYY represented 10% of the  
 total binding.

### Short Circuit Current Measurements

The antisecretory effects of the peptides were  
 investigated by measuring the short-circuit current (SCC)  
 35 in rat jejunal mucosa mounted in a Ussing chamber and  
 automatically voltage clamped as described by Cox et al.  
*(J. Physiol. supra)*. Briefly, strips of mucosa were  
 placed between two halves of perspex Ussing chambers

- 19 -

(window size, 0.6 cm<sup>2</sup>) containing oxygenated (95% O<sub>2</sub>/5% CO<sub>2</sub>) Krebs-Henseleit solution (NaCl, 117 mM, KCl 4.7 mM, CaCl<sub>2</sub>, 2.5 mM; MgSO<sub>4</sub> 1.2 mM, NaHCO<sub>3</sub> 24.8 mM and glucose 11.1 mM), pH 7.4, 37°C. Routinely, four preparations of  
5 jejunum were obtained from each animal and these exhibited comparable potential differences and SCC, but they were not paired. Preparations were automatically voltage clamped using a W-P dual voltage clamp and the SCC displayed continuously on pen recorders. Once a  
10 stable baseline SCC was reached, peptides were added to the basolateral reservoir only, and cumulative concentration-response profiles constructed.

#### Data Analyses

All points in the binding experiments are the mean  
15 of at least three experiments performed in duplicate. For clarity, the SEMs in the binding experiments are not shown in Fig. 2, but were less than 10%. Values of changes in SCC are quoted as  $\mu\text{A}/0.6\text{cm}^2$  mean  $\pm$  1 SEM from between 3 and 7 different preparations. EC<sub>50</sub> values were  
20 calculated from pooled cumulative concentration - response curves using an iterative curve fitting program. Comparison of data groups (SCC recordings) were made using unpaired Student's t-tests where a p value <0.5 was considered statistically significant.

25 There now follows the results of the biological activities of the compounds of the invention (see Table 1 and Table 2). As described below, the tested compounds were assayed for purity and for their binding and antisecretory potencies in rat jejunum.

30 Purified peptides were found to be > 96% homogeneous by analytical reversed phase chromatography and, in addition, had the expected amino acid composition and masses. For example, Fig. 1 shows the RP-HPLC chromatogram of N- $\alpha$ -Ac-[Phe<sup>27</sup>]PYY(22-36)(SEQ. ID. NO. 3).  
35 The free peptides were further characterized by sequence

- 20 -

analysis (see, Table 1 and Table 2). The overall yields of the peptides were in the range of 10% to 30%.

PYY, [im-DNP-His<sup>26</sup>]PYY (SEQ. ID. NO. 9) and the analogs of PYY(22-36) (SEQ. ID. NO. 10) displaced <sup>125</sup>I-PYY bound to rat jejunal epithelial plasma membranes in a concentration-dependent manner. Although [im-DNP-His<sup>26</sup>]PYY (SEQ. ID. NO. 9) and PYY(22-36) (SEQ. ID. NO. 10) were 20-times less potent than PYY based on IC<sub>50</sub> values, they displayed the same maximal response as the intact hormone (Fig. 2, Table 1). Substitution of Thr<sup>32</sup> with Ala as in [Ala<sup>32</sup>]PYY(22-36) (SEQ. ID. NO. 11) resulted in the lowering of the binding potency while the replacement of both Ser<sup>23</sup> and Thr<sup>32</sup> with Ala further reduced the receptor affinity. Also, introduction of a negative charge at position 28 without altering the helicity as in [Glu<sup>28</sup>]PYY(22-36) (SEQ. ID. NO. 13) decreased the binding possibly due to the disruption of the ionic interactions. Since the hydrophobic groups are known to increase the interaction with the receptors (Balasubramaniam et al. *Biochem. Biophys. Res. Comm.* 137:1041, 1986), the binding of a N- $\alpha$ -myristoyl- and N- $\alpha$ -naphthaleneacetyl-derivatives of PYY(22-36) was also determined. Both these analogs exhibited slightly lower binding affinity than PYY(22-36) (SEQ. ID. NO. 10) possibly due to increased steric hinderance. On the other hand, N- $\alpha$ -acetylation of PYY(22-36) (SEQ. ID. NO. 14) increased the receptor affinity four times. Further structure-activity studies with N- $\alpha$ -Ac-PYY(22-36) (SEQ. ID. NO. 20) revealed that substitution of Tyr<sup>36</sup> with N-Me-Tyr or His<sup>26</sup> with p.Cl-Phe lowers the binding potency. However, replacement of Tyr<sup>27</sup> with Phe increased the receptor affinity by 28%. As a control, the binding of PYY(22-36) (SEQ. ID. NO. 10) and several of its analogs were also tested. However, none of these analogs inhibited the binding of <sup>125</sup>I-PYY even at 10  $\mu$ M.

- 21 -

In mucosal preparations of rat jejunum PYY(22-36) (SEQ. ID. NO. 10) analogs reduced the baseline SCC in a concentration dependent manner (Fig. 3A and B) and calculated EC<sub>50</sub> values are listed in Table 1. The PYY(22-36) (SEQ. ID. NO. 10) analogs were generally less potent as antisecretory agents than as inhibitors of binding. The order of analog potency was similar to that from binding studies with two notable exceptions, namely N- $\alpha$ -myristoyl-PYY(22-36) (SEQ. ID. NO. 18) and N- $\alpha$ -naphthaleneacetyl-PYY(22-36) (SEQ. ID. NO. 19). N- $\alpha$ -acetylation and substitution of Tyr<sup>27</sup> with Phe increased the antisecretory potency of PYY(22-36) and this analog, N- $\alpha$ -Ac-[Phe<sup>27</sup>] PYY(22-36) (SEQ. ID. NO. 3), was only 9-times less potent than the intact hormone. Furthermore, there was no significant difference between the maximal inhibitory responses, these being - 12.6 $\pm$ 2.4 and - 12.0 $\pm$ 1.3  $\mu$ A/0.6cm<sup>2</sup> for PYY (440 nM, n = 6) (SEQ. ID. NO. 1) and N- $\alpha$ -Ac-[Phe<sup>27</sup>] PYY(22-36) (1.4  $\mu$ M, n = 7) (SEQ. ID. NO. 3), respectively.

TABLE 1: Comparison of the binding and antiseecretory potencies of PYY, PYY fragments and their analogs

5	PEPTIDES	RT <sup>a</sup>	MH <sup>+</sup> (Calc.)	BINDING <sup>b</sup> (min)	SCC <sup>b</sup>	IC <sub>50</sub> (nM)	EC <sub>50</sub> (nM)
	PYY (SEQ. ID. NO. 1)			4.8	4240.2 (4241.7)	0.2	1.7
	NPY (SEQ. ID. NO. 24)			34.0 <sup>c</sup>	4253.8 (4254.7)	2.0	9 <sup>d</sup>
	[Im-DNP-His <sup>26</sup> ]PYY (SEQ. ID. NO. 9)			8.7 <sup>c</sup>	4406.9 (4407.8)	4.0	72
10	PYY(22-36) (SEQ. ID. NO. 10)			4.4	1888.8 (1890.2)	4.0	77
	[Ala <sup>32</sup> ]PYY(22-36) (SEQ. ID. NO. 11)			4.7	1858.8 (1860.2)	71	n.d.
	[Ala <sup>23,32</sup> ]PYY(22-36) (SEQ. ID. NO. 12)			4.3	1842.8 (1844.2)	>10,000	n.d.
	[Glu <sup>28</sup> ]PYY(22-36) (SEQ. ID. NO. 13)			3.8	1905.1 (1906.2)	199	n.d.
	N- $\alpha$ -Ac-PYY(22-36) (SEQ. ID. NO. 14)			10.0	1930.9 (1932.2)	1.12	40
15	N- $\alpha$ -Ac-[p.ClPhe <sup>26</sup> ]PYY(22-36) (SEQ. ID. NO. 15)			14.9 <sup>c</sup>	1975.4 (1976.7)	50	124
	N- $\alpha$ -Ac-[Glu <sup>28</sup> ]PYY(22-36) (SEQ. ID. NO. 16)			3.9	1947.0 (1948.2)	44.7	3,000
20	N- $\alpha$ -Ac-[N-Me-Tyr <sup>36</sup> ]PYY(22-36) (SEQ. ID. NO. 17)			13.5	1945.3 (1946.3)	354	792
	N- $\alpha$ -Ac-[Phe <sup>27</sup> ]PYY(22-36) (SEQ. ID. NO. 3)			8.3	1915.3 (1916.2)	0.80	15.1
	N- $\alpha$ -Myristoyl-PYY(22-36) (SEQ. ID. NO. 18)			4.8	2099.0 (2100.6)	17.8	3,300
25	N- $\alpha$ -Naphthaleneacetyl-PYY(22-36) (SEQ. ID. NO. 19)			17.0	2056.9 (2058.4)	8.9	19,500

a: isocratic, 27% CH<sub>3</sub>CN containing 0.1% TFA; b: mean of three separate experiments;  
c: isocratic, 32% CH<sub>3</sub>CN containing 0.1% TFA; d: from reference 10; n.d.: not determined



- 23 -

N- $\alpha$ -myristoyl-PYY(22-36) (SEQ. ID. NO. 18) and N- $\alpha$ -naphthaleneacetyl-PYY(22-36) (SEQ. ID. NO. 19) analogs, in contrast to their moderate binding potency, exhibited poor antisecretory responses with threshold concentrations of about 20nM and EC<sub>50</sub> values greater than 2 and 30  $\mu$ M respectively. After a cumulative concentration of 7.4  $\mu$ M, N- $\alpha$ -myristoyl-PYY(22-36) (SEQ. ID. NO. 18) reduced the basal SCC by  $-5.2 \pm 0.6 \mu\text{A}/0.6\text{cm}^2$  (n = 7). Subsequent addition of PYY (100 nM) further reduced the SCC by  $-10.2 \pm 0.7 \mu\text{A}/0.6\text{cm}^2$  (n = 7) and this was not significantly different from control responses to PYY(22-36) (SEQ. ID. NO. 10) could antagonize PYY responses, three tissues were treated with the analog (1 $\mu$ M) and PYY concentration-response curves were constructed and compared with controls. The fragment reduced the basal current by  $-0.4 \pm 0.3 \mu\text{A}/0.6\text{cm}^2$  and the resultant PYY EC<sub>50</sub> value ( $4.4 \pm 1.2$  nM, n = 3) did not differ significantly from that of the nontreated controls ( $2.6 \pm 1.1$  nM, n = 3).

These results show that modification of the active site of PYY (SEQ. ID. NO. 1), PYY(22-36) (SEQ. ID. NO. 10), can lead to a substantial increase in both the binding and antisecretory potencies of this fragment. The key analogs in this series exhibited the following order of potency: PYY (SEQ. ID. NO. 1) > N- $\alpha$ -Ac-[Phe<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 3) > N- $\alpha$ -Ac-PYY(22-36) (SEQ. ID. NO. 14) > PYY(22-36) (SEQ. ID. NO. 10). Furthermore, our investigations revealed that the hydroxyl groups of Ser<sup>23</sup> and Thr<sup>32</sup> as well as the imidazole group of His<sup>26</sup> are important for interaction with intestinal PYY-preferring receptors. Although there was, in general, a good correlation between the binding and antisecretory potencies of the analogs, there were also notable exceptions.

- 24 -

N- $\alpha$ -myristoyl-PYY(22-36) (SEQ. ID. NO. 18) and N- $\alpha$ -naphthaleneacetyl-PYY(22-36) (SEQ. ID. NO. 19) analogs inhibited  $^{125}\text{I}$ -PYY binding with moderate potency, but exhibited poor antisecretory responses. This observation suggested that these analogs may be antagonists. However, prior pretreatment of jejunal membranes with these analogs failed to significantly alter the antisecretory responses to PYY and the reason for the discrepancy remains unclear at present.

10 Table 2 and Fig. 4 present the  $\text{IC}_{50}$  values for additional PYY(22-36) (SEQ. ID. NO. 10) and PYY (25-36) analogs. Based on the results presented in Table 2 the analogs in this series exhibited the following order of potency:

15 N- $\alpha$ -Ac-[Tic $^{27}$ ]PYY(22-36) (SEQ. ID. NO. 25) < N- $\alpha$ -Ac-[Bip $^{27}$ ]PYY(22-36) (SEQ. ID. NO. 22) < N- $\alpha$ -Ac-[Nal $^{27}$ ]PYY(22-36) (SEQ. ID. NO. 23) < N- $\alpha$ -Ac-[Bth $^{27}$ ]PYY(22-36) (SEQ. ID. NO. 21) < N- $\alpha$ -Ac-[Phe $^{27}$ ]PYY(22-36) (SEQ. ID. NO. 3) < N- $\alpha$ -Ac-[Phe $^{27}$ ]PYY(25-  
20 36) (SEQ. ID. NO. 26) < N- $\alpha$ -Ac-[Trp $^{27}$ ]PYY(22-36) (SEQ. ID. NO. 5) < N- $\alpha$ -Ac-[Thi $^{27}$ ]PYY(22-36) (SEQ. ID. NO. 6) < N- $\alpha$ -Ac-PYY(22-36) (SEQ. ID. NO. 14) < PYY (SEQ. ID. NO. 1).

- 25 -

TABLE 2 Comparison of Receptor Binding Data for PYY and PYY analogs

PEPTIDE NO.	Peptide Structure	IC <sub>50</sub> (nM)
	PYY (SEQ. ID. NO. 1)	0.04
	N- $\alpha$ -Ac-PYY(22-36) (SEQ. ID. NO. 14)	0.08
905	N- $\alpha$ -Ac-[Bth <sup>27</sup> ]PYY(22-36) (SEQ. ID. NO. 21)	0.22
5 906	N- $\alpha$ -Ac-[Bip <sup>27</sup> ]PYY(22-36) (SEQ. ID. NO. 22)	4.46
911	N- $\alpha$ -Ac-[Nal <sup>27</sup> ]PYY(22-36) (SEQ. ID. NO. 23)	0.39
915	N- $\alpha$ -Ac-[Trp <sup>27</sup> ]PYY(22-36) (SEQ. ID. NO. 5)	0.10
916	N- $\alpha$ -Ac-[Thi <sup>27</sup> ]PYY(22-36) (SEQ. ID. NO. 6)	0.095
914	N- $\alpha$ -Ac-[Phe <sup>27</sup> ]PYY(25-36) (SEQ. ID. NO. 26)	0.15
10 913	N- $\alpha$ -Ac-[Tic <sup>27</sup> ]PYY(22- 36) (SEQ. ID. NO. 25)	4.50

NPY/PYY receptors characterized to date have been broadly classified into Y-1, Y-2 and Y-3 subtypes (Balsubramaniam et al. *J. Biol. Chem.* 265:14724, 1990; Michel, *Trends Pharmacol. Sci.* 12:389, 1991). Both Y-1 and Y-2 receptors exhibit a preference for PYY over NPY, and more significantly C-terminal fragments of NPY and PYY are effective only at the Y-2 subtype. Y-3 receptors, on the other hand, exhibit a greater affinity for NPY than PYY. Since rat jejunal mucosa antisecretory responses show an order of agonist potency PYY (SEQ. ID. NO. 1) > NPY (SEQ. ID. NO. 24) > PYY(13-36) (SEQ. ID. NO. 32) > NPY(13-36) (SEQ. ID. NO. 33) these epithelial receptors are Y-2 like, and are completely insensitive to the Y-1 selective agonist [Pro<sup>34</sup>]NPY (Cox et al. *Peptides*, *supra*). The results further describe N- $\alpha$ -Ac-PYY(22-36)

- 26 -

(SEQ. ID. NO. 14) and N- $\alpha$ -Ac-[Phe<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 3) to be more potent than PYY(22-36) (SEQ. ID. NO. 10) and the corresponding C-terminal fragments of NPY of varying lengths (Cox et al. *Br. J. Pharmacol. supra*).

5 The higher affinity for PYY (SEQ. ID. NO. 1) and its C-terminal fragments compared with NPY (SEQ. ID. NO. 24) and its respective fragments is in agreement with the order of potency obtained from receptor binding studies with rat intestinal epithelial membranes (Laburthe et al. *supra*; Laburthe, *supra*; Voisin et al. *Ann. N.Y. Acad. Sci. supra*; Voisin et al. *Am. J. Physiol.* )

In addition, analogs listed in Table 3 were synthesized as described above and tested for binding activity. The results shown in Table 3 demonstrate that

15 N- $\alpha$ -Ac-[Tyr<sup>22</sup>, Phe<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 7) is similar in its competitive binding as PYY (SEQ. ID. NO. 1), indicating that the introduction of an aromatic amino acid, e.g., Tyr, at position 22 is an effective PYY analog.

20. TABLE 3

PEPTIDE NO.	Peptide Structure	IC <sub>50</sub> (nM)
	PYY (SEQ. ID. NO. 1)	0.10
917	N- $\alpha$ -Ac-[Phe <sup>27</sup> , Thi <sup>36</sup> ]PYY(22-26) (SEQ. ID. NO. 27)	4.46
918	N- $\alpha$ -Ac-[Thz <sup>26</sup> , Phe <sup>27</sup> ]Pyy(22-36) (SEQ. ID. NO. 28)	4.50
904	N- $\alpha$ -Ac-[Pcp <sup>27</sup> ]PYY(22-36) (SEQ. ID. NO. 29)	1.58
25 908	N- $\alpha$ -Ac-[Phe <sup>22,27</sup> ]PYY(22-36) (SEQ. ID. NO. 30)	11.22
910	N- $\alpha$ -Ac-[Tyr <sup>22</sup> , Phe <sup>27</sup> ]PYY(22-36) (SEQ. ID. NO. 7)	0.10

- 27 -

USE

In the practice of the method of the present invention, an effective amount of an any one or combination of the analogs of the invention, e.g., N- $\alpha$ -  
5 Ac-[Phe<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 3), N- $\alpha$ -Ac-[Trp<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 24), N- $\alpha$ -Ac-[Phe<sup>27</sup>]PYY(25-36) (SEQ. ID. NO. 3), N- $\alpha$ -Ac-[Thi<sup>27</sup>]PYY(22-36) (SEQ. ID. NO. 6) or derivative thereof, is administered via any of the usual and acceptable methods known in the art, either  
10 singly or in combination with another compound or compounds of the present invention. These compounds or compositions can thus be administered orally (e.g., buccal cavity), sublingually, parenterally (e.g., intramuscularly, intravenously, or subcutaneously),  
15 rectally (e.g., by suppositories or washings), transdermally (e.g., skin electroporation) or by inhalation (e.g., by aerosol), and in the form of either solid, liquid or gaseous dosage, including tablets and suspensions. The administration can be conducted in a  
20 single unit dosage form with continuous therapy or in a single dose therapy ad libitum.

Thus, the method of the present invention is practiced when relief of symptoms is specifically required or perhaps imminent. Alternatively, the method  
25 of the present invention is effectively practiced as continuous or prophylactic treatment.

Useful pharmaceutical carriers for the preparation of the compositions hereof, can be solids, liquids or gases; thus, the compositions can take the form of  
30 tablets, pills, capsules, suppositories, powders, enterically coated or other protected formulations (e.g. binding on ion-exchange resins or packaging in lipid-protein vesicles), sustained release formulations, solutions, suspensions, elixirs, aerosols, and the like.  
35 The carrier can be selected from the various oils

- 28 -

including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, and the like. Water, saline, aqueous dextrose, and glycols are preferred liquid carriers, particularly (when isotonic with the blood) for injectable solutions. For example, formulation for intravenous administration comprise sterile aqueous solutions of the active ingredient(s) which are prepared by dissolving solid active ingredient(s) in water to produce an aqueous solution, and rendering the solution sterile. Suitable pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, talc, gelatin, malt, rice, flour, chalk, silica, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The compositions may be subjected to conventional pharmaceutical additives such as preservatives, stabilizing agents, wetting or emulsifying agents, salts for adjusting osmotic pressure, buffers and the like. Suitable pharmaceutical carriers and their formulation are described in *Remington's Pharmaceutical Sciences* by E.W. Martin. Such compositions will, in any event, contain an effective amount of the active compound together with a suitable carrier so as to prepare the proper dosage form for proper administration to the recipient.

The dose of the compound of the present invention for treating the above-mentioned disorders varies depending upon the manner of administration, the age and the body weight of the subject, and the condition of the subject to be treated, and ultimately will be decided by the attending physician or veterinarian. Such amount of the active compound as determined by the attending physician or veterinarian is referred to herein as a "therapeutically effective amount". Thus, a typical

- 29 -

administration is oral administration or parenteral administration. The daily dose in the case of oral administration is typically in the range of 0.1 to 100 mg/kg body weight, and the daily dose in the case of  
5 parenteral administration is typically in the range of 0.001 to 50 mg/kg body weight.

To be effective for the prevention or treatment of gastroenterological disorders, especially infectious (e.g. viral or bacterial) or inflammatory diarrhea, or  
10 diarrhea resulting from surgery, it is important that the therapeutic agents be relatively non-toxic, non-antigenic and non-irritating at the levels in actual use.

It is understood that the examples and embodiments described herein are for illustrative purposes only and  
15 that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

- 30 -

## SEQUENCE LISTING

## (1) GENERAL INFORMATION

- (i) APPLICANT: UNIVERSITY OF CINCINNATI
- (ii) TITLE OF THE INVENTION: ANALOGS OF PEPTIDE YY AND USES THEREOF
- (iii) NUMBER OF SEQUENCES: 30
- (iv) CORRESPONDENCE ADDRESS:  
 (A) ADDRESSEE: Fish & Richardson P.C.  
 (B) STREET: 225 Franklin Street  
 (C) CITY: Boston  
 (D) STATE: MA  
 (E) COUNTRY: US  
 (F) ZIP: 02110-2804
- (v) COMPUTER READABLE FORM:  
 (A) MEDIUM TYPE: Diskette  
 (B) COMPUTER: IBM Compatible  
 (C) OPERATING SYSTEM: DOS  
 (D) SOFTWARE: FastSEQ Version 2.0
- (vi) CURRENT APPLICATION DATA:  
 (A) APPLICATION NUMBER:  
 (B) FILING DATE: 13-NOV-1996  
 (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:  
 (A) NAME: Tsao, Y. Rocky  
 (B) REGISTRATION NUMBER: 34,476  
 (C) REFERENCE/DOCKET NUMBER: 00537/105WO1
- (ix) TELECOMMUNICATION INFORMATION:  
 (A) TELEPHONE: 617-542-5070  
 (B) TELEFAX: 617-542-8906  
 (C) TELEX:

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 36 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: N/A  
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Tyr	Pro	Ala	Lys	Pro	Glu	Ala	Pro	Gly	Glu	Asp	Ala	Ser	Pro	Glu	Glu
1				5				10					15		
Leu	Ser	Arg	Tyr	Tyr	Ala	Ser	Leu	Arg	His	Tyr	Leu	Asn	Leu	Val	Thr
			20				25					30			
Arg	Gln	Arg	Tyr												
			35												

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 36 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: N/A



- 31 -

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Tyr	Pro	Ile	Lys	Pro	Glu	Ala	Pro	Gly	Glu	Asp	Ala	Ser	Pro	Glu	Glu
1				5					10					15	
Leu	Asn	Arg	Tyr	Tyr	Ala	Ser	Leu	Arg	His	Tyr	Leu	Asn	Leu	Val	Thr
			20					25					30		
Arg	Gln	Arg	Tyr												
			35												

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 15 is an abbreviation of N-Me-Tyr. The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ala	Ser	Leu	Arg	His	Phe	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Xaa
1				5					10					15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ala	Ser	Leu	Arg	His	Phe	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5					10					15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single

- 32 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(ix) FEATURE:

(D) OTHER INFORMATION: The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala	Ser	Leu	Arg	His	Trp	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5					10					15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: N/A

(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 6 is an abbreviation of Thi (2-thienylalanine). The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala	Ser	Leu	Arg	His	Xaa	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5					10					15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: N/A

(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Tyr	Ser	Leu	Arg	His	Phe	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5					10					15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

- 33 -

(B) TYPE: amino acid  
 (C) STRANDEDNESS: N/A  
 (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Arg His Phe Leu Asn Leu Val Thr Arg Gln Arg Tyr  
 1 5 10

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: N/A  
 (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 26 is an abbreviation of im-DNP-His. The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Tyr Pro Ala Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu  
 1 5 10 15  
 Leu Ser Arg Tyr Tyr Ala Ser Leu Arg Xaa Tyr Leu Asn Leu Val Thr  
 20 25 30  
 Arg Gln Arg Tyr  
 35

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: N/A  
 (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr  
 1 5 10 15

## (2) INFORMATION FOR SEQ ID NO:11:

- 34 -

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ala	Ser	Leu	Arg	His	Tyr	Leu	Asn	Leu	Val	Ala	Arg	Gln	Arg	Tyr
1				5					10					15

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ala	Ala	Leu	Arg	His	Tyr	Leu	Asn	Leu	Val	Ala	Arg	Gln	Arg	Tyr
1				5					10					15

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ala	Ser	Leu	Arg	His	Tyr	Glu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5					10					15

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- 35 -

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr  
1                      5                      10                      15

## (2) INFORMATION FOR SEQ ID NO:15:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 5 is an abbreviation of p.Cl.Pro. The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ala Ser Leu Arg Xaa Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr  
1                      5                      10                      15

## (2) INFORMATION FOR SEQ ID NO:16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ala Ser Leu Arg His Tyr Glu Asn Leu Val Thr Arg Gln Arg Tyr  
1                      5                      10                      15

## (2) INFORMATION FOR SEQ ID NO:17:

- 36 -

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 15 is an abbreviation of N-Me-Tyr. The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala	Ser	Leu	Arg	His	Tyr	Glu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Xaa
1				5					10					15

## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: The sequence has a myristoylated N-terminus (i.e., N- $\alpha$ -myristoyl), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ala	Ser	Leu	Arg	His	Tyr	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5					10					15

## (2) INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: The sequence has a naphthalenoacetyl N-terminus (i.e., N- $\alpha$ -naphthaleneacetyl), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ala	Ser	Leu	Arg	His	Tyr	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5					10					15

- 37 -

## (2) INFORMATION FOR SEQ ID NO:20:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala	Ser	Leu	Arg	His	Tyr	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5				10					15	

## (2) INFORMATION FOR SEQ ID NO:21:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 6 is an abbreviation of Bth. The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ala	Ser	Leu	Arg	His	Xaa	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5				10					15	

## (2) INFORMATION FOR SEQ ID NO:22:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

## (ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 6 is an abbreviation of Bip. The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ala	Ser	Leu	Arg	His	Xaa	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

**1                      5                      10                      15**

(A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: N/A  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

(D) OTHER INFORMATION: Xaa in position 6 is an abbreviation of Tic. The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The



- 39 -

sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ala	Ser	Leu	Arg	His	Xaa	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5				10					15	

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

His	Phe	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5				10		

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 15 is an abbreviation of Thi. The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Ala	Ser	Leu	Arg	His	Phe	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Xaa
1				5				10					15	

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

(ix) FEATURE:

- 40 -

(D) OTHER INFORMATION: Xaa in position 15 is an abbreviation of Thz. The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala	Ser	Leu	Arg	Xaa	Phe	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5					10					15

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 6 is an abbreviation of Pcp. The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ala	Ser	Leu	Arg		Xaa	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5					10					15

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: N/A
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: The sequence has an acetylated N-terminus (i.e., N- $\alpha$ -Ac), rather than an amino N-terminus (i.e., H<sub>2</sub>N-). The sequence has an amide C-terminus (i.e., CO-NH<sub>2</sub>), rather than a carboxyl C-terminus (i.e., CO-OH).

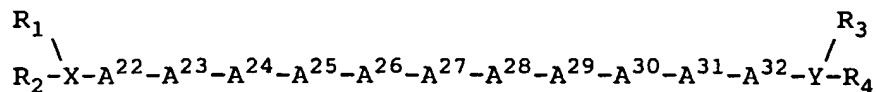
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Phe	Ser	Leu	Arg		Phe	Leu	Asn	Leu	Val	Thr	Arg	Gln	Arg	Tyr
1				5					10					15

What is claimed is:

- 41 -

1. A compound having the formula:



5 wherein

X is Cys or is deleted;

Y is a chain of 0-4 amino acids, inclusive,  
the C-terminal one of which is bonded to R<sub>3</sub> and R<sub>4</sub>;

10 R<sub>1</sub> is H, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>6</sub>-C<sub>18</sub> aryl, C<sub>1</sub>-C<sub>12</sub> acyl,  
C<sub>7</sub>-C<sub>18</sub> aralkyl, or C<sub>7</sub>-C<sub>18</sub> alkaryl;

R<sub>2</sub> is H, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>6</sub>-C<sub>18</sub> aryl, C<sub>1</sub>-C<sub>12</sub> acyl,  
C<sub>7</sub>-C<sub>18</sub> aralkyl, or C<sub>7</sub>-C<sub>18</sub> alkaryl;

A<sup>22</sup> is an aromatic amino acid, Ala,

Aib, Anb, N-Me-Ala, or is deleted;

15 A<sup>23</sup> is Ser, Thr, Ala, Aib, N-Me-Ser, N-Me-Thr, N-  
Me-Ala, D-Trp, or is deleted;

A<sup>24</sup> is Leu, Gly, Ile, Val, Trp, Nle, Nva, Aib,

Anb,

N-Me-Leu, or is deleted;

20 A<sup>25</sup> is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-  
ε-NH-R (where R is H, a branched or straight  
chain C<sub>1</sub>-C<sub>10</sub> alkyl group, or an aryl group),  
Orn or is deleted;

25 A<sup>26</sup> is Ala, His, Thr, 3-Me-His, 1-Me-His,  
β-pyrozolylalanine, N-Me-His, Arg, Lys, homo-  
Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is  
H, a branched chain or straight chain C<sub>1</sub>-C<sub>10</sub>  
alkyl group, or an aryl group), Orn, or is  
deleted;

30 A<sup>27</sup> is an aromatic amino acid other than Tyr;

A<sup>28</sup> is Leu, Val, Trp, Nle, Nva, Aib, Anb, or  
N-Me-Leu;

A<sup>29</sup> is Asn, Ala, Gln, Gly, Trp, or N-Me-Asn;

A<sup>30</sup> is Leu, Ile, Val, Trp, Nle, Nva, Aib, Anb, or

- 42 -

N-Me-Leu;

A<sup>31</sup> is Val, Leu, Ile, Trp, Nle, Nva, Aib, Anb, or  
N-Me-Val;

A<sup>32</sup> is Thr, Ser, N-Me-Ser, N-Me-Thr, or D-Trp;

5 R<sub>3</sub> is H, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>6</sub>-C<sub>18</sub> aryl, C<sub>1</sub>-C<sub>12</sub> acyl, C<sub>7</sub>-  
C<sub>18</sub> aralkyl, or C<sub>7</sub>-C<sub>18</sub> alkaryl; and

R<sub>4</sub> is H, C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>6</sub>-C<sub>18</sub> aryl, C<sub>1</sub>-C<sub>12</sub> acyl, C<sub>7</sub>-  
C<sub>18</sub> aralkyl, C<sub>7</sub>-C<sub>18</sub> alkaryl, or a  
pharmaceutically acceptable salt thereof.

10 2. The compound of claim 1, wherein A<sup>27</sup> is Phe,  
Nal, Bip, Pcp, Tic, Trp, Trp, Bth, Thi, or Dip.

3. The compound of claim 1, where Y is A<sup>33</sup>-A<sup>34</sup>-  
A<sup>35</sup>-A<sup>36</sup> wherein

15 A<sup>33</sup> is Cys, Arg, Lys, homo-Arg, diethyl-homo-Arg,  
Lys-ε-NH-R (where R is H, a branched or  
straight chain C<sub>1</sub>-C<sub>10</sub> alkyl group, or C<sub>6</sub>-C<sub>18</sub>  
aryl group), or Orn;

A<sup>34</sup> is Cys, Gln, Asn, Ala, Gly, N-Me-Gln, Aib, or  
Anb;

20 A<sup>35</sup> is Cys, Arg, Lys, homo-Arg, diethyl-homo-Arg,  
Lys-ε-NH-R (where R is H, a branched or  
straight chain C<sub>1</sub>-C<sub>10</sub> alkyl group, or C<sub>6</sub>-C<sub>18</sub>  
aryl group), or Orn; and

25 A<sup>36</sup> is an aromatic amino acid, Cys, or a  
pharmaceutically acceptable salt thereof.

4. The compound of claim 3, wherein said compound  
has the formula:

N-α-Ac-Ala-Ser-Leu-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-  
Gln-Arg-Tyr-NH<sub>2</sub> (SEQ. ID. NO. 3), or a pharmaceutically  
30 acceptable salt thereof.

- 43 -

5. The compound of claim 3, wherein said compound has the formula:

H-Ala-Ser-Leu-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH<sub>2</sub> (SEQ. ID. NO. 4), or a pharmaceutically

5 acceptable salt thereof.

6. The compound of claim 3, wherein said compound has the formula:

N- $\alpha$ -Ac-Ala-Ser-Leu-Arg-His-Trp-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH<sub>2</sub> (SEQ. ID. NO. 5), or a pharmaceutically

10 acceptable salt thereof.

7. The compound of claim 3, wherein said compound has the formula:

N- $\alpha$ -Ac-Ala-Ser-Leu-Arg-His-Thi-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH<sub>2</sub> (SEQ. ID. NO. 6), or a pharmaceutically

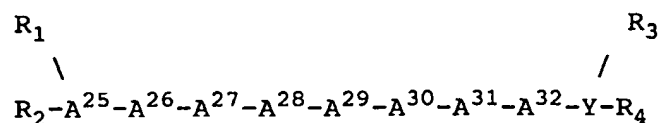
15 acceptable salt thereof.

8. The compound of claim 3, wherein said compound has the formula:

N- $\alpha$ -Ac-Tyr-Ser-Leu-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-NH<sub>2</sub> (SEQ. ID. NO. 7), or a

20 pharmaceutically acceptable salt thereof.

9. A compound having the formula:



25 wherein

the N-terminal amino acid is bonded to R<sub>1</sub> and R<sub>2</sub>;

Y is a chain of 0-4 amino acids, inclusive the C-terminal one of which is bonded to R<sub>3</sub> and R<sub>4</sub>;

- 44 -

- $R_1$  is H,  $C_1$ - $C_{12}$  alkyl,  $C_6$ - $C_{18}$  aryl,  $C_1$ - $C_{12}$  acyl,  
 $C_7$ - $C_{18}$  aralkyl, or  $C_7$ - $C_{18}$  alkaryl;  
 $R_2$  is H,  $C_1$ - $C_{12}$  alkyl,  $C_6$ - $C_{18}$  aryl,  $C_1$ - $C_{12}$  acyl,  
 $C_7$ - $C_{18}$  aralkyl, or  $C_7$ - $C_{18}$  alkaryl;  
5  $A^{25}$  is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-  
 $\epsilon$ -NH-R (where R is H, a branched or straight  
chain  $C_1$ - $C_{10}$  alkyl group, or an aryl group),  
Orn or is deleted;  
 $A^{26}$  is Ala, His, Thr, 3-Me-His,  
10  $\beta$ -pyroazolylalanine, N-Me-His, Arg, Lys, homo-  
Arg, diethyl-homo-Arg, Lys- $\epsilon$ -NH-R (where R is  
H, a branched or straight chain  $C_1$ - $C_{10}$  alkyl  
group, or an aryl group), or Orn;  
 $A^{27}$  is an aromatic amino acid;  
15  $A^{28}$  is Leu, Val, Trp, Nle, Nva, Aib, Anb, or  
N-Me-Leu;  
 $A^{29}$  is Asn, Ala, Gln, Gly, Trp, or N-Me-Asn;  
 $A^{30}$  is Leu, Ile, Val, Trp, Nle, Nva, Aib, Anb, or  
N-Me-Leu;  
20  $A^{31}$  is Val, Leu, Ile, Trp, Nle, Nva, Aib, Anb, or  
N-Me-Val;  
 $A^{32}$  is Thr, Ser, N-Me-Ser, N-Me-Thr, or D-Trp;  
 $R_3$  is H,  $C_1$ - $C_{12}$  alkyl,  $C_6$ - $C_{18}$  aryl,  $C_1$ - $C_{12}$  acyl,  $C_7$ -  
 $C_{18}$  aralkyl, or  $C_7$ - $C_{18}$  alkaryl; and  $R_4$  is H,  
25  $C_1$ - $C_{12}$  alkyl,  $C_6$ - $C_{18}$  aryl,  $C_1$ - $C_{12}$  acyl,  
 $C_7$ - $C_{18}$  aralkyl or  $C_7$ - $C_{18}$  alkaryl, or a  
pharmaceutically acceptable salt thereof.

10. The compound of claim 9, wherein  $A^{27}$  is Phe, Nal, Bip, Pcp, Tic, Trp, Bth, Thi, or Dip.

- 45 -

11. The compound of claim 9, wherein Y is A<sup>33</sup>-A<sup>34</sup>-A<sup>35</sup>-A<sup>36</sup> wherein
- 5           A<sup>33</sup> is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-  
ε-           NH-R (where R is H, a branched or straight  
            chain C<sub>1</sub>-C<sub>10</sub> alkyl group, or C<sub>6</sub>-C<sub>18</sub> aryl  
            group), Cys, or Orn;
- A<sup>34</sup> is Cys, Gln, Asn, Ala, Gly, N-Me-Gln, Alb, or  
            Anb;
- 10           A<sup>35</sup> is Arg, Lys, homo-Arg, diethyl-homo-Arg, Lys-  
ε-           NH-R (where R is H, a branched or straight  
            chain C<sub>1</sub>-C<sub>10</sub> alkyl group, or C<sub>6</sub>-C<sub>18</sub> aryl  
            group), Cys, or Orn; and
- A<sup>36</sup> is an aromatic amino acid, Cys, or a  
            pharmaceutically acceptable salt thereof.

12. The compound of claim 11, wherein said  
compound has the formula:  
N-α-Ac-Arg-His-Phe-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr-  
NH<sub>2</sub> (SEQ. ID. NO. 26), or a pharmaceutically acceptable  
salt thereof.

13. A therapeutic composition capable of  
decreasing excess intestinal water and electrolyte  
secretion, said composition comprising a therapeutically  
effective amount of the compound of claim 1 or claim 9,  
together with a pharmaceutically acceptable carrier  
25 substance.

14. A method of decreasing excess intestinal  
water and electrolyte secretion in a mammal, said method  
comprising administering to said mammal a therapeutically  
effective amount of the composition of claim 1 or claim  
30 9, together with a pharmaceutically acceptable carrier  
substance.

- 46 -

15. A method of regulating cell proliferation in a mammal, said method comprising administering to said mammal a therapeutically effective amount of the composition of claim 1 or claim 9, together with a  
5 pharmaceutically acceptable carrier substance.

16. A method of augmenting nutrient transport in a mammal, said method comprising administering to said mammal a therapeutically effective amount of the composition of claim 1 or claim 9, together with a  
10 pharmaceutically acceptable carrier substance.

17. A method of regulating lipolysis in a mammal, said method comprising administering to said mammal a therapeutically effective amount of the composition of claim 1 or claim 9, together with a pharmaceutically  
15 acceptable carrier substance.

18. A method of regulating blood flow in a mammal, said method comprising administering to said mammal a therapeutically effective amount of the composition of claim 1 or claim 9, together with a  
20 pharmaceutically acceptable carrier substance.

19. A dimeric compound comprising either two peptides of claim 1 or claim 9, or one peptide of claim 1 or one peptide of claim 9, wherein said dimer is formed by either an amide bond, or a disulfide bridge between  
25 said two peptides.



1/5

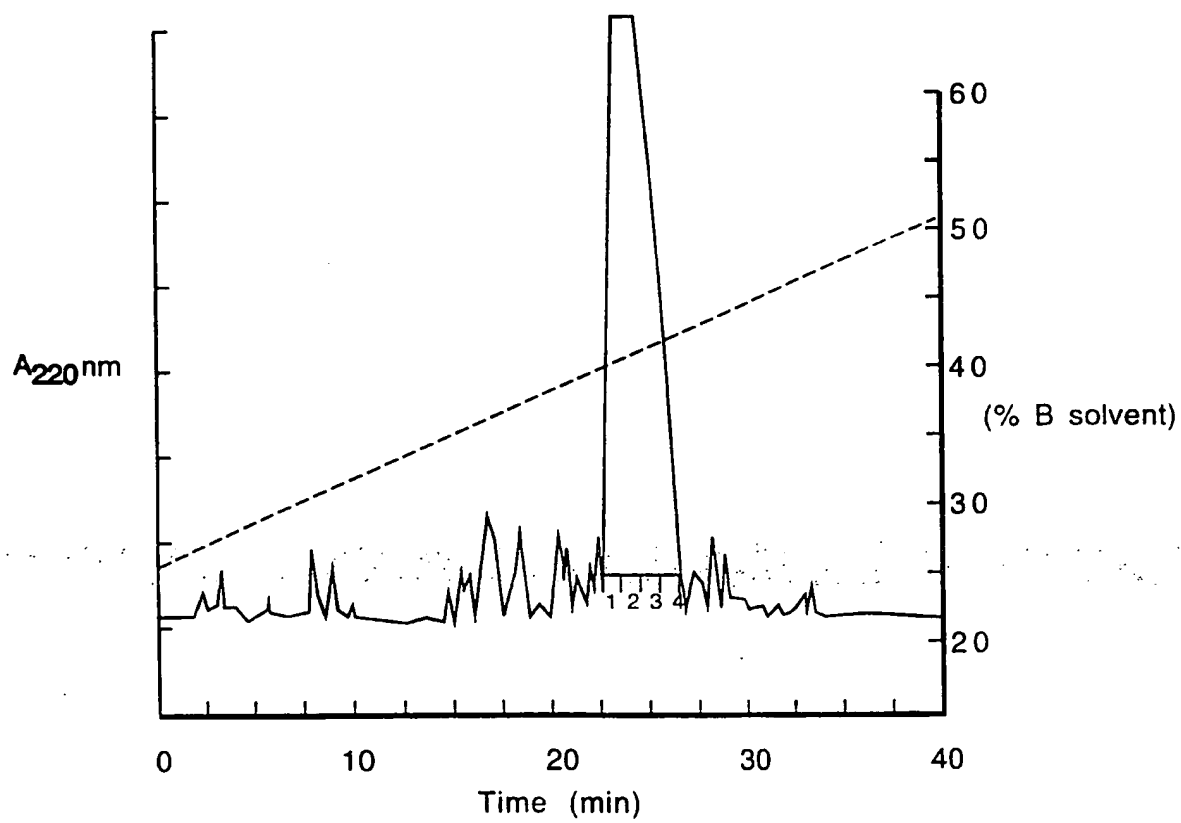


FIG. 1

2/5

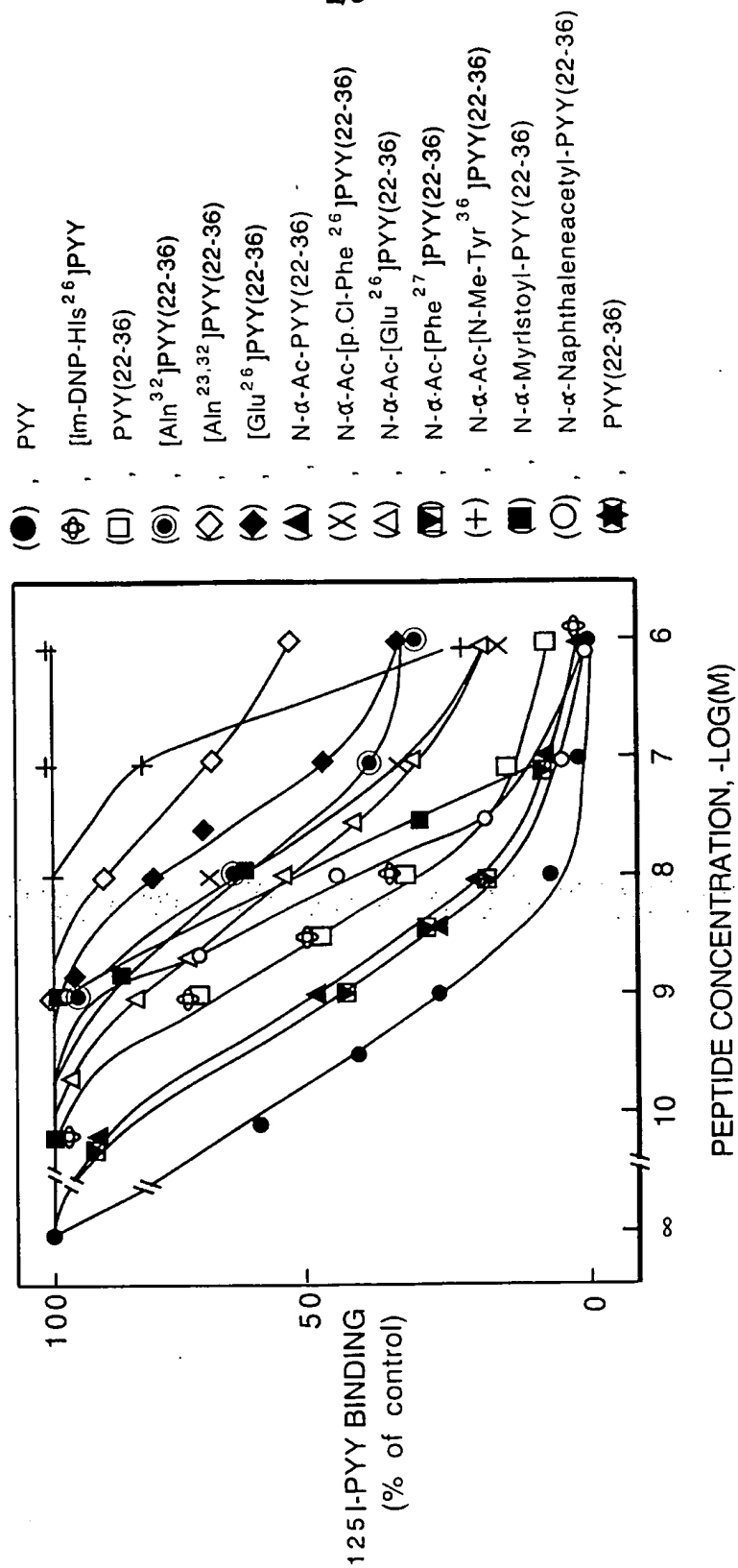


FIG. 2

3/5

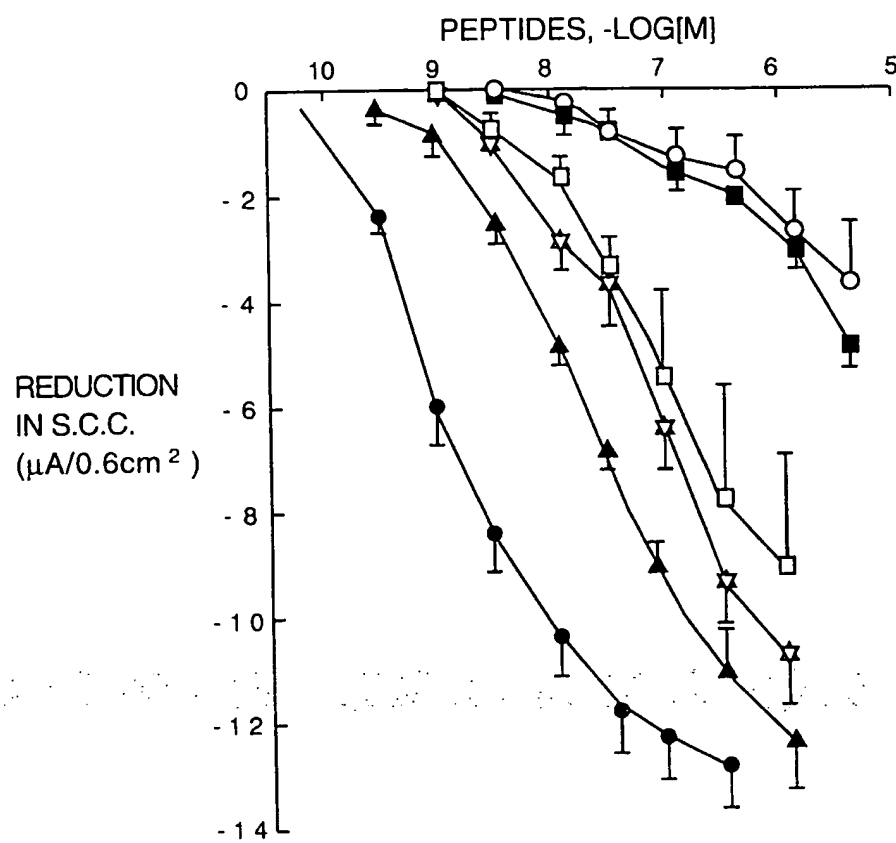


FIG. 3A

4/5

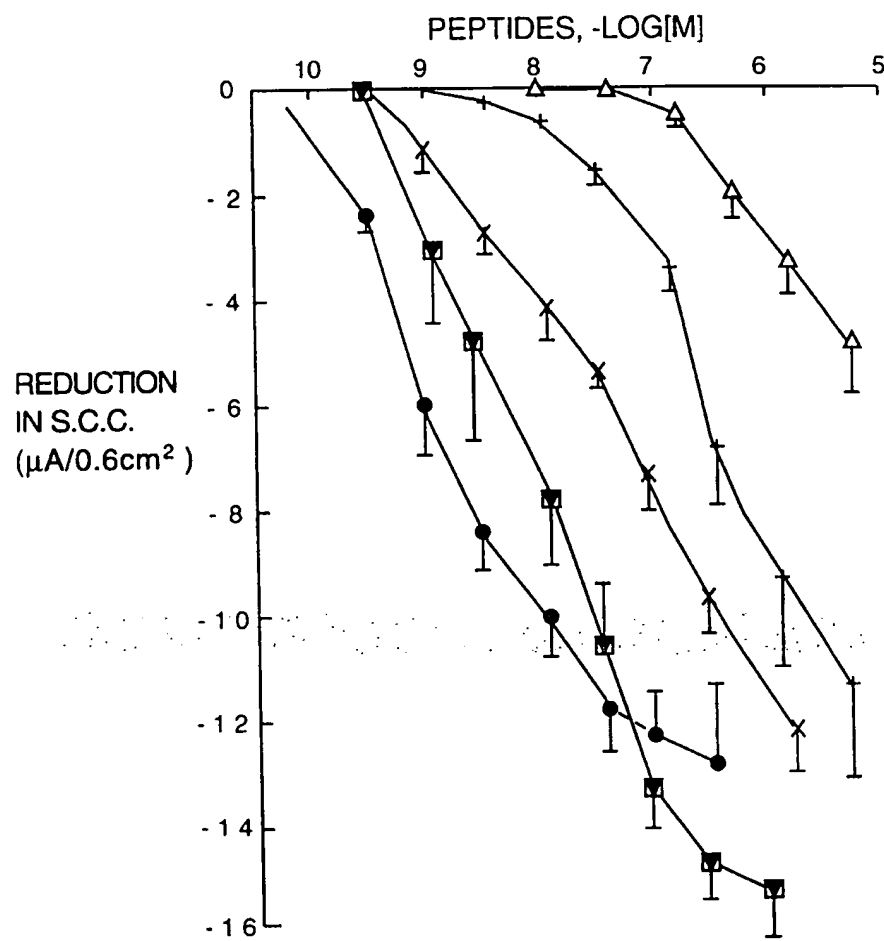


FIG. 3B

5/5

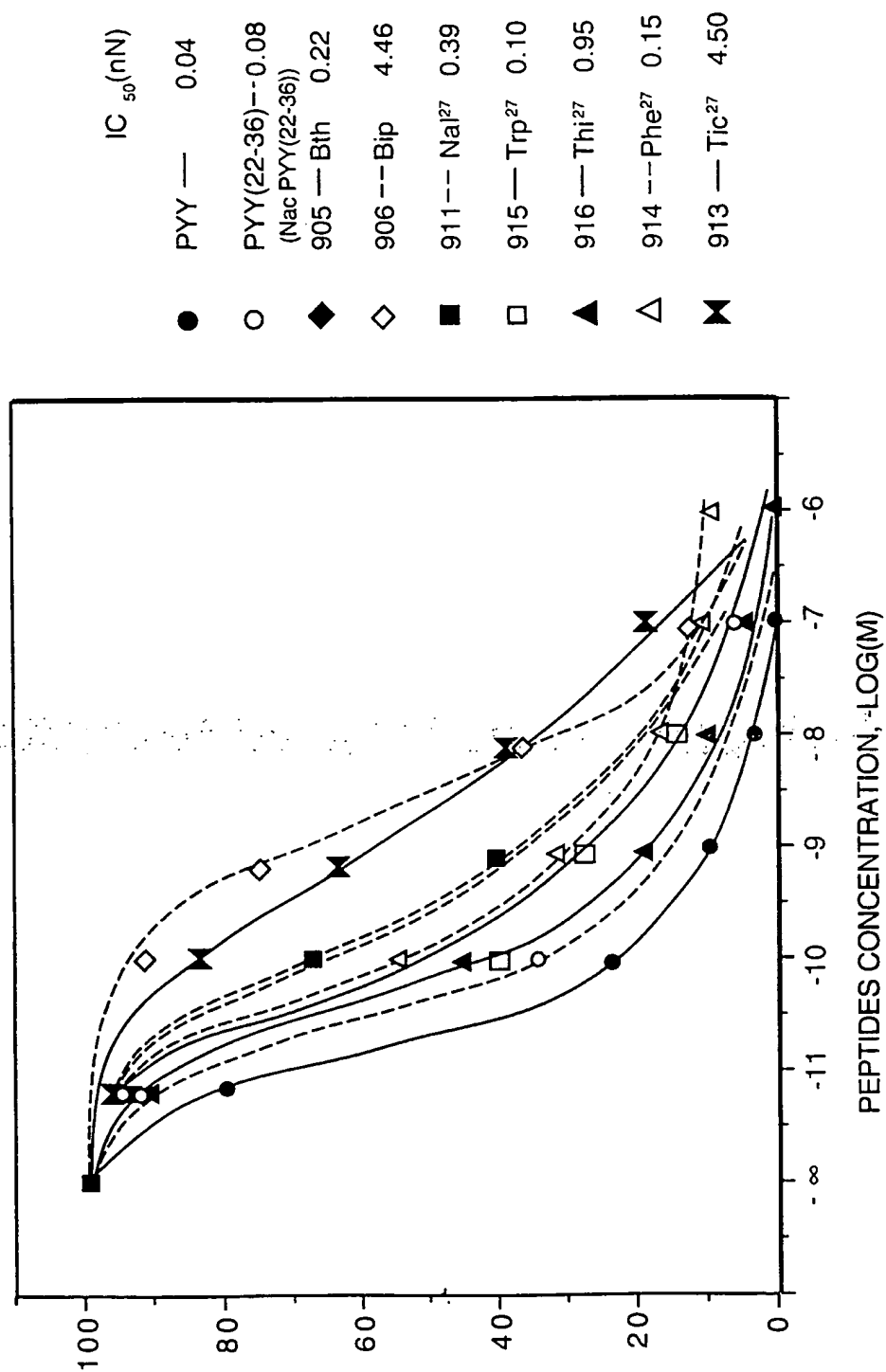


FIG. 4

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/18374**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :A61K 38/00; C07K 5/00, 7/00, 17/00

US CL :514/12, 14, 15; 530/324, 326, 327

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 14, 15; 530/324, 326, 327.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, STN, BIOSIS, MEDLINE, EMBASE.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,328,899 A (BOUBLIK et al.) 12 July 1994, see entire document.	1-19
Y	US 5,026,685 A (BOUBLIK et al.) 25 June 1991, see entire document.	1-19
Y	TATEMOTO et al. Synthesis of receptor antagonists of neuropeptide Y. Proc. Natl. Acad. Sci., USA. February 1992, Vol 89, pages 1174-1178, see entire document.	1-19
Y	TATEMOTO, K. Neuropeptide Y and Its Receptor Antagonists: Use of an Analog Mixture-Screening Strategy. Annals New York Academy of Sciences. 1990, Vol 611, pages 1-5, see entire document.	1-19

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

03 FEBRUARY 1997

Date of mailing of the international search report

14 MAR 1997

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

AVIS M. DAVENPORT

Telephone No. (703) 308-0196

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☒ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**